Atmos. Chem. Phys., 3, 1703–1708, 2003
www.atmos-chem-phys.org/acp/3/1703/

Atmospheric Chemistry and Physics

Effect of water addition and nitrogen fertilization on the fluxes of CH_4, CO_2, NO_x, and N_2O following five years of elevated CO_2 in the Colorado Shortgrass Steppe

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Received: 28 January 2003 – Published in Atmos. Chem. Phys. Discuss.: 20 May 2003
Revised: 12 August 2003 – Accepted: 28 August 2003 – Published: 13 October 2003

Abstract. An open-top-chamber (OTC) CO_2 enrichment ($\sim$720 μmol mol^{-1}) study was conducted in the Colorado shortgrass steppe from April 1997 through October 2001. Aboveground plant biomass increased under elevated CO_2 and soil moisture content was typically higher than under ambient CO_2 conditions. Fluxes of CH_4, CO_2, NO_x and N_2O, measured weekly year round were not significantly altered by CO_2 enrichment over the 55 month period of observation. During early summer of 2002, following the removal of the open-top-chambers from the CO_2 enrichment sites in October 2001, we conducted a short term study to determine if soil microbial processes were altered in soils that had been exposed to double ambient CO_2 concentrations during the growing season for the past five years. Microplots were established within each experimental site and 10 mm of water or 10 mm of water containing the equivalent of 10 g m^{-2} of ammonium nitrate-N was applied to the soil surface. Fluxes of CO_2, CH_4, NO_x and N_2O fluxes within control (unchambered), ambient CO_2 and elevated CO_2 OTC soils were measured at one to three day intervals for the next month. With water addition alone, CO_2 and NO emission did not differ between ambient and elevated CO_2 soils, while CH_4 uptake rates were higher and N_2O fluxes lower in elevated CO_2 soils. Adding water and mineral N resulted in increased CO_2 emissions, increased CH_4 uptake and decreased NO emissions in elevated CO_2 soils. The N addition study confirmed previous observations that soil respiration is enhanced under elevated CO_2 and N immobilization is increased, thereby decreasing NO emission.

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1 Introduction

During the past few decades the atmospheric concentration of CO_2 has increased at historically unprecedented rates, as have N_2O and CH_4 concentrations (IPCC, 2001). Increasing CO_2 concentrations will have a direct effect on plant production and plant communities and indirectly feed back into a number of soil biotic systems that influence long term ecosystem viability (Hungate et al., 1997a, b, c; Owensby et al., 1993a). The impact of elevated CO_2 on the shortgrass steppe, which is used extensively for grazing and is similar to regions which occupy about 8% of the U.S. and about 11% of global land area (Bailey, 1979) has not been previously addressed. These interactive feedbacks on the soil C and N cycles and their influence on trace gas fluxes have potentially important impacts on the global atmospheric budgets of the gases and the long term sustainability of the grassland. Earlier studies within the shortgrass steppe have demonstrated that such grasslands play an important role as consumers of atmospheric CH_4, and producers of N_2O (Mosier et al., 1991; 1996; 1997). Doubling CO_2 had little impact on trace gas fluxes in the shortgrass steppe (Morgan et al., 2001; Mosier et al., 2002a). The few measurements of NO_x, N_2O, CH_4 and CO_2 fluxes in CO_2 enrichment studies in other ecosystems have given contradictory results.

Growth chamber studies suggest that plant C/N ratios, nitrogen use efficiency and water use efficiency all increase under elevated CO_2 (Drake et al., 1996; Morgan et al., 1994; Rogers et al., 1994). In the short term, increases in soil moisture content resulting from higher water use efficiency (Hungate et al., 1997a, b, c) may accelerate rates of C and N mineralization , increasing N availability for plant uptake. Over the long term, however, decreased litter quality is expected to increase N immobilization rates and reduce N availability for plant uptake. From these observations we hypothesized that initially elevated CO_2 would induce increased soil

Table 1. Selected soil properties* of the soil at the Open-Top-Chamber study site

Soil Depth (cm)	Sand	Silt	Clay	pH	Total N	Total C	Bulk Density
	%				%		$g\,cm^{-3}$
0–10	76	14	10	7.3	0.101	0.891	1.28
10–20	74	15	11	8.1	0.077	0.606	1.42
20–30	74	13	13	8.0	0.076	0.584	1.48

*Gravimetric soil water content for the 0–15 cm depth at water filled pore space of 0.2, 0.4 and 0.6 is 7.2, 14.4 and 21.6 $kg\,kg^{-1}$, respectively (Mosier et al., 1998).

moisture and increased N mineralization rates (Hunt et al., 1988). As a result, CO_2, NO and N_2O emissions should increase on the short term under elevated CO_2 , while CH_4 uptake should decrease. Over the longer term, however, increased C/N ratios in plant litter and roots would result in longer term decreases in N mineralization rates, and decreased NO and N_2O fluxes would be observed. To test these responses, Mosier et al., (2002a) monitored the soil-atmosphere exchange of CO_2, NO_x, N_2O and CH_4 weekly, year-round, April 1997 to November 2001 on unchambered control, ambient CO_2 and $\sim$720 $\mu mol\,mol^{-1}$ CO_2 experimental plots in the Colorado shortgrass steppe. Mosier et al., (2002a, b) observed no statistically significant CO_2 enrichment effect on ecosystem respiration, oxidation of atmospheric CH_4, or emissions of NO_x or N_2O. Methane oxidation tended to be higher under elevated CO_2 while NO_x and N_2O tended to be lower, but not significantly in either case. Above ground biomass production was higher under elevated CO_2 (Morgan et al., 2001), which utilized more soil N (King et al., 2003). However, soil N mineralization was probably enhanced under elevated CO_2 because of more moist soils (Hungate et al., 1997a, b, c). The two opposing processes apparently offset each other because NO_x and N_2O emissions, which reflect system N mineralization and nitrification, did not differ. Ecosystem respiration, which included soil and aboveground plant respiration, was not generally higher under elevated CO_2 (Mosier et al., 2002a). During the 5 years of the study trace gas exchange measurements suggested that soil microbial processes were not greatly altered under double CO_2 concentrations, at least in the short term. Plant production during a year of above average rainfall did, however, appear to be limited by N availability (King et al., 2003). By analyzing the concentration of soil CO_2 at different depths in the OTCs and calculating soil respiration, Pendall et al., (2003) found that elevated CO_2 increased soil respiration by about 25% in a moist growing season and by about 85% in a dry season. Significant increases in soil respiration rates occurred only during dry periods. $\delta^{13}C$ analyses of soil CO_2 revealed that soil organic matter decomposition rates were more than doubled under elevated CO_2 whereas rhizosphere respiration rates were not changed. Estimates of net ecosystem production, which account for both inputs and losses of carbon, suggest that soil carbon sequestration is not increased under elevated CO_2 during dry years, but may be in wet years (Pendall et al., accepted).

We were interested to determine if residual effects on microbial processes persisted following CO_2 enrichment. During early summer of the year following the removal of the open- top chambers from the CO_2 enrichment sites we conducted a short term study to determine if soil microbial processes were altered in soils that had been exposed to double ambient CO_2 concentrations during the growing season for the past five years. The response of emissions of CO_2, NO_x and N_2O and the uptake of atmospheric CH_4 to water addition and water and mineral nitrogen fertilization to soils that had or had not been exposed to elevated CO_2 are reported in this paper.

2 Materials and Methods

The CO_2 enrichment studies were conducted at the USDA/ARS Central Plains Experimental Range (CPER) on which is located the Shortgrass Steppe long-term-ecological-research (LTER) project, about 60 km NE of Fort Collins, CO, USA (40°50' N, 104°42' W). The semiarid grassland site is at 1650 m elevation and has a long term average annual precipitation of $\sim$320 mm with mean summer air temperatures of 15.6°C and 0.6°C mean winter air temperatures (Morgan et al., 2001). Annual mineral nitrogen input is an estimated 0.5 $g\,N\,m^{-2}$ mainly through wet and dry atmospheric deposition (Mosier et al., 1996).

Experimental Site

In the fall of 1995, vegetation and soil surveys were conducted in native shortgrass steppe at the experimental site. The survey results enabled a selection of relatively similar experimental plots on the basis of soil and plant community information and documented plot differences before the treatments were implemented. The grassland community is comprised of over 25 species of forbs and grasses, but dominated by three grass species, Bouteloua gracilis (C_4, accounts for approximately 42% of total

aboveground biomass), Pascopyrum smithii (C_3, 21% of total aboveground biomass) and Stipa comata (C_3, 26% of total aboveground biomass). After 5-years of exposure to double ambient CO_2 concentrations the plant species composition had changed to approximately 50% Stipa comata (Morgan et al., 2003). Nine experimental plots of similar plant species composition were selected on the basis of this initial survey. The soil within the study site is a Remmit fine sandy loam (Ustollic camborthids) which holds 18% (gravimetric) water at field capacity, and 4% at the permanent wilting point (Table 1).

On six of the nine plots, open-top chambers (4.5 m diameter by 3 m height) of similar design to Owensby et al., (1993a, b) were installed in March 1997 (Morgan et al., 2001a). All chambers were equipped with blowers to exchange ~1.5 air volumes per minute. Three of the chambers had precision valve outlets located in-line between the blowers and the chambers and attached to a compressed CO_2 source for elevating CO_2 to approximately 720 mol mol^{-1}. Carbon dioxide in the chambers was controlled only during the growing season, from early April to late October. Chambers were removed in the winter. Daily precipitation was measured at a meteorological station located about 50 m from the OTCs. The CO_2 enrichment was terminated in late October of 2001.

Establishment of Microplots and Trace Gas Flux Measurements

In late April, 2002 two microplots and gas flux sampling locations were established by driving flux chamber anchors (20 cm diameter, 10 cm high PVC cylinder) 8 cm into the soil within each OTC and control (unchambered) plot. In the morning of 11 June 2002, water (1-cm) or water plus N-fertilizer (1-cm of water containing the equivalent of 10 g N m^{-2} of ammonium nitrate) was added to each microplot. Soil moisture was the same in all treatments at the beginning of this study and emissions of all trace gases were small at this time. We measured fluxes of NO_x, N_2O, CH_4 and CO_2, two hours after water addition and then at one to three day intervals until 12 July 2002. Measurements were typically made midmorning of each sampling day using vented closed chambers (Hutchinson and Mosier, 1981; Mosier et al., 1991) where the changes in concentration of N_2O, CO_2 and CH_4 within the chamber were measured by withdrawing samples from the chamber by syringe at three time periods and analyzing the gas concentrations by gas chromatography (Mosier et al., 1996; 1997; 1998). Nitric oxide flux was monitored from the same chamber anchors on the same day using a flow-through chamber system (Martin et al., 1998) and a Thermo Environmental Instruments model 42C chemiluminescence NO-NO_2-NO_x analyzer that is housed in the instrument trailer (Mosier et al., 1998). NO_x emissions from the soil are typically >90% NO, so fluxes will generally be discussed in terms of NO only (Martin et al., 1998; Mosier et al., 1998). Air and soil temperatures were monitored at each flux measurement using a hand held digital thermometer.

Statistical Analyses

Gas flux measurements, soil moisture and soil temperatures within each treatment replicate (OTC or unchambered location) were averaged (n=3) for each observation time. Over the month long analysis period the eleven flux measurements were averaged and single factor analysis of variance was performed (Microsoft Excel). Individual CO_2 treatments were compared for a designated time interval using a paired t-test. Significance levels of 0.05 were used unless specifically noted.

3 Results and Discussion

Trace Gas Fluxes

During the five year study prior to the N-addition experiment NO_x flux averaged 4.3 in ambient and 4.1 μg N m^{-2} hr^{-1} in elevated chambers . NO_x flux was negatively correlated to plant biomass production. Nitrous oxide emission rates averaged 1.8 and 1.7 μg N m^{-2} hr^{-1}, CH_4 flux rates averaged -31 and -34 μg C m^{-2} hr^{-1} and ecosystem respiration averaged 43 and 44 mg C m^{-2} hr^{-1} under ambient and elevated CO_2, respectively, over the same time period (Mosier et al., 2002a, b). We conducted the short-term N-addition to see if any residual effects of elevated CO_2 remained during the growing season following the five years of CO_2 fumigation and found that some important changes in soil microbial responses resulting from CO_2 enrichment.

NO and N_2O Emissions

Under the hot and very dry conditions at the study site when this study was initiated NO and N_2O emissions are typically very low (Mosier et al., 1998). NO emissions from the control soils were higher than from soils that had been under ambient or CO_2 enriched chambers when the soils were irrigated (Table 2). This response was similar to the long term NO fluxes observed where control soil NO emissions averaged 11 μg m^{-2} hr^{-1} compared to 4.3 and 4.1 in ambient and CO_2 enriched chambers (Mosier et al., 2002a). Plant production and uptake of nitrogen was opposite this trend with plant biomass being greater under elevated CO_2>ambient CO_2>unchambered control (Morgan et al., 2001). This suggests that N availability for NO production by nitrifiers is partly regulated by plant uptake (Mosier et al., 2002a). Nitrous oxide emissions were significantly lower ($P<0.05$) from soils that had been exposed to elevated CO_2 (Table 2). This may also reflect the lower N availability to soil microbes under elevated CO_2 due

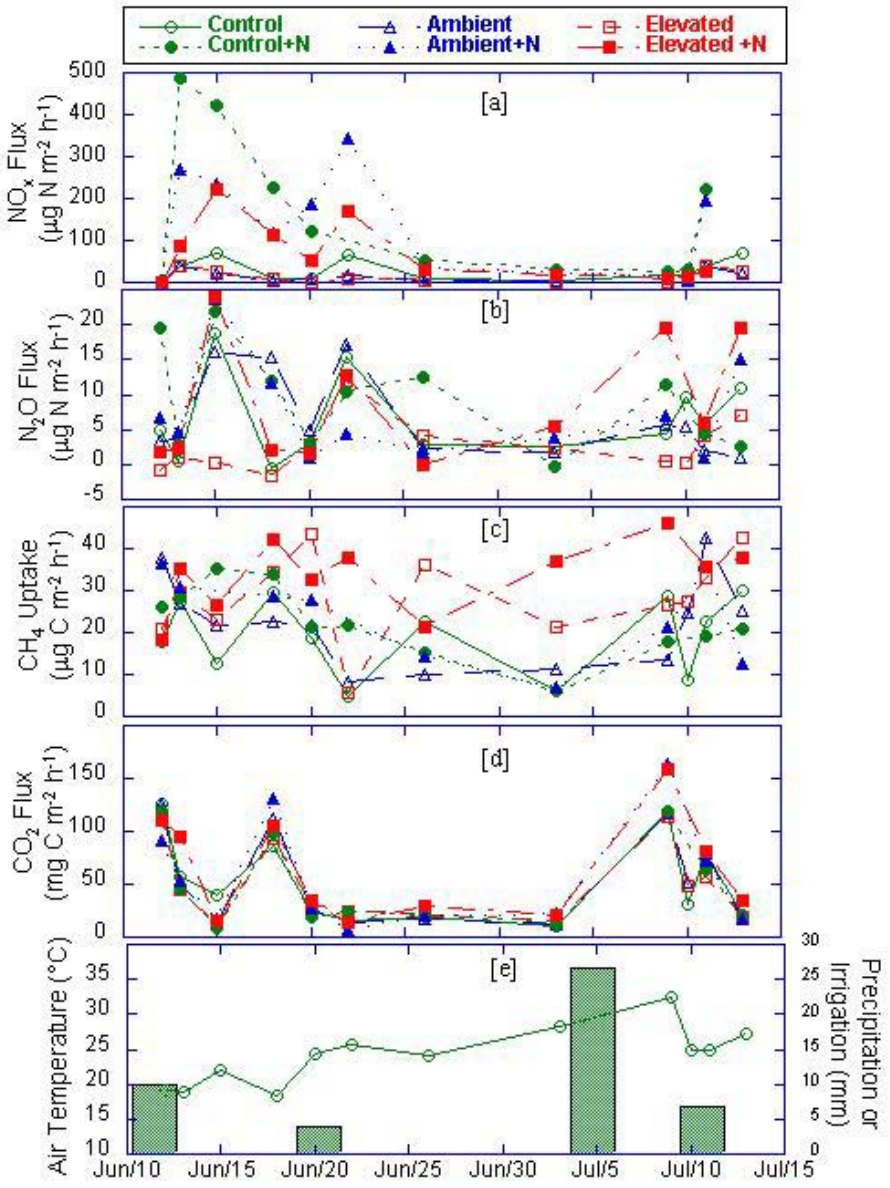

Fig. 1. Trace gas flux, air temperature and soil water content in ambient and elevated CO_2 chambers: **(a)** NO_x flux; **(b)** N_2O flux; **(c)** uptake of atmospheric CH_4 by soil micro-organisms; **(d)** dark chamber CO_2 flux which includes above ground plant respiration, plant root respiration and soil microbial respiration; **(e)** air temperature at time of flux measurements and water addition and precipitation.

to enhanced plant production (King et al., 2003). Soil mineral N concentrations were typically very low (<1 mg/kg) in all treatments (Mosier, unpublished data). Both N_2O and to a lesser extent NO emissions increased following water application (Figs. 1a and b). Over the measurement period when water alone was added, NO/N_2O ratios ranged between 2 and 5 with the highest in the elevated CO_2 soils.

When NH_4NO_3 was added N_2O flux tripled in elevated CO_2 soils ($P<0.05$), and increased slightly in control and ambient chamber soils ($P>0.05$). NO emissions and N_2O emissions in N-fertilized soils increased markedly following each irrigation and precipitation event (Figs. 1a, b, and e). Nitric oxide fluxes increased almost 10 fold with N addition in control and ambient CO_2 soils but only about 5-fold in elevated CO_2 soils. NO emissions were significantly lower from elevated CO_2 soils than from control or ambient CO_2 soils, again indicating the lower availability of N in the elevated CO_2 soils. Hungate et al., (1997b, c) found that, during wet up, NO emissions were depressed by 55% in high nutrient conditions under elevated CO_2 (ambient+360 μmol mol^{-1}) while there was no difference among treatments in N_2O emissions. They attributed the

Table 2. Mean mean trace gas flux rates within the shortgrass steppe open-top-chamber CO_2 enrichment study area the summer following 5-years of CO_2 enrichment, 11 June–12 July 2002

CO_2 Treatment	CO_2 $mg\,C\,m^{-2}\,hr^{-1}$	CH_4 $\mu g\,C\,m^{-2}\,hr^{-1}$	NO_x $\mu g\,N\,m^{-2}hr^{-1}$	N_2O
Water Only				
Control	52a	−19b	25a	6.5a
Ambient	53a	−22b	13b	6.6a
High	49a	−28a	13b	2.6b
Water + N				
Control	50b	−22b	160a	9.1a
Ambient	57b	−24ab	121a	7.4a
High	64a	−34a	63b	8.6a

*Numbers in each column followed by the same letter are not significantly different ($P>0.05$).

decreased NO emissions under elevated CO_2 to increased N immobilization. Increased utilization of added N by soil microbes, thus a decrease in NO emissions, appears to be the case in this study as well (Table 2).

CO_2 and CH_4 Fluxes

Plant growth during the time of the study was virtually nonexistant because of the very low amount of precipitation that had fallen in the preceding year. Ecosystem CO_2 flux (dark chamber respiration which includes plant, root and soil microbial respiration) increases following water addition were similar in all soils (Fig. 1d; Table 2). Only with water+N addition did CO_2 fluxes from elevated CO_2 soils exceed those from control and ambient soils ($P<0.05$). Microbial respiration appears to be enhanced under elevated CO_2 (Pendall et al., 2003), especially when microbes are not limited by water or N availability. N addition appeared to stimulate soil microbial respiration while decreasing NO emissions because of increased microbial immobilization of added N. Hu et al., (2001) suggest that over the long term, soil microbial decomposition is slowed under elevated CO_2 because of N limitation. The rate of uptake of atmospheric CH_4 was significantly greater ($P<0.05$) in elevated CO_2 soils than either control or ambient CO_2 soils (Fig. 1c; Table 2). CH_4 uptake rates were not measurably enhanced with N addition in control or ambient CO_2 soils but tended to be greater in elevated CO_2 soils ($P>0.05$). During the 5-years of CO_2 enrichment CH_4 uptake rates tended to be higher under elevated CO_2. This short term study suggests that a microbial population developed under elevated CO_2 which tended to increase utilization of atmospheric CH_4. Ineson et al., (1998) observed lower CH_4 uptake rates under elevated CO_2 within a free-atmosphere CO_2-enrichment (FACE) study in Switzerland. They also observed lower CO_2 respiration rates and increased N_2O emissions under elevated CO_2. McLain et al., (2002) also observed lower CH_4 consumption rates under elevated CO_2 in a pine plantation. The decrease in CH_4 consumption was attributed, in part to wetter soils under elevated CO_2. Soil conditions in the pine forest were likely much more comparable to the grassland soils in Switzerland (Ineson et al., 1998) than to the much drier conditions in the Colorado shortgrass steppe. The wetter soil conditions under elevated CO_2 in the Colorado grassland (Ferretti et al., 2003) likely produced more favorable conditions for methanotrophic activity, rather than limiting CH_4 diffusion into the soil in the Swiss grassland (Ineson et al., 1998) and the pine forest (McLain et al., 2002).

Summary

In a semi-arid shortgrass steppe, addition of water and ammonium nitrate to soils that had been exposed to double-ambient CO_2 concentrations during the growing season of the previous five years increased ecosystem respiration and atmospheric CH_4 oxidation and decreased NO emissions. These observations suggest that methanotroph populations were enhanced under elevated CO_2 while soil N supply was depleted by increased plant growth (King et al., 2003; Morgan et al., 2001). Soil respiration was higher in elevated CO_2 soils following irrigation and N addition, suggesting that microbes were becoming N limited. Although decomposition rates were twice as high under elevated as ambient CO_2 mid-way through the 5-year experiment (Pendall et al., 2003), the N fertilization response observed here suggests that eventually microbial decomposition rates will slow, as predicted by Hu et al., (2001), leading to increased C sequestration potential.

Acknowledgements. We thank David Jensen who collected the trace gas data. We also thank the Terrestrial Ecology and Climate Change Initiative grants (NSF-IBN-9524068 and USDA/NRICGP-98-134), NSF DEB-9708596 for funding support, for SGS-LTER that provided partial salary support for David Jensen and USDA-ARS which provided the remaining support for this project.

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